Electrophoretic Analysis of Ribosomal and Viral Ribonucleic Acids with a Simple Technique for Slicing Low-Concentration Polyacrylamide Gels

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The electrophoretic mobilities of ribosomal ribonucleic acids (RNA) from cultured mammalian (HeLa, Vero, MDBK), avian (chick embryo), and bacterial (*Escherichia coli*) cells, and RNA species extracted from selected viruses (Sindbis, polio, tobacco mosaic, Sendai) were compared, employing a simple, inexpensive technique for slicing low-concentration polyacrylamide gels. The procedure provides for rapid fractionation of gels used for characterization of RNA, incorporating extrusion and serial sectioning of frozen gels. Among 28S ribosomal RNA species, Vero and MDBK were indistinguishable, whereas HeLa RNA had a slightly lower mobility (higher apparent molecular weight) and chick RNA had a higher mobility (lower apparent molecular weight). The 18S ribosomal RNA species of the three mammalian sources were indistinguishable, but chick 18S RNA had a slightly lower apparent molecular weight. The inverse relation between mobility and log-molecular weight among the ribosomal and viral RNA species, though not highly precise, demonstrates the applicability of the technique to the study of molecular weights of viral RNA species.

Gel electrophoresis is known to separate ribonucleic acids (RNA) not readily separable by centrifugation. Polyacrylamide gels have proven very useful for fractionation of RNA and, based on a linear relation between relative mobility and log-molecular weight, for estimations of molecular weight of RNA (1, 15–18). Gels of low concentration (2 to 3% acrylamide) employed in electrophoresis of high-molecular-weight RNA lack rigidity and are difficult to handle. Some investigators incorporate agarose to stiffen the gels (8, 18). Others use a dialysis membrane to retain the soft gel in vertical tube electrophoresis, and freeze the gel for sectioning after removal from the tube (1, 6). A variation employs freezing in situ, with subsequent removal of the frozen gel from the tube for sectioning (8). Our procedure incorporates the latter technique, employing a novel apparatus in which the frozen gel is extruded and serially sectioned.

Ribosomal RNA species from cultured cells and RNA species from selected viruses were characterized by employing our procedure for both analytical and micropreparative techniques. Coelectrophoresis of RNA species, usually employing two radioisotopes, permitted us to confirm the finding of Loening (15) of an apparent

difference in molecular weight between chick and mammalian 28S ribosomal RNA species and to refine and extend comparisons among mammalian 28S RNA species. In addition, we detected a difference in electrophoretic mobility between 18S ribosomal RNA species from chick and mammalian cells. (For purposes of identification, the various categories of ribosomal RNA are referred to by their nominal S values without implying that precise sedimentation coefficients have been determined.)

MATERIALS AND METHODS

Preparation of RNA. The sources of cellular RNA species were *Escherichia coli* (^{32}P-labeled cells kindly provided by Maurice Bondurant), primary chick embryo cultures, and the following lines in continuous culture: HeLa (human), Vero (African green monkey), and MDBK (bovine). The sources of viral RNA species were poliovirus (strain LSc-2ab propagated in Vero cells), Sindbis virus (propagated in Vero cells), Sendai virus (propagated in MDBK cells, employing embryonated egg-passaged stock virus), and tobacco mosaic virus (TMV; ^{3}H-labeled RNA kindly provided by H. Fraenkel-Conrat and B. Singer). Conditions for culturing the vertebrate cells, propagating viruses, and radioactive labeling (uri-

dine-5-^{3}H, 2 μCi/ml, or ^{32}P-orthophosphate, 20 μCi/ml) were those usually employed in this laboratory (11, 12, 21, 22). Uninfected cells were scraped into the medium and pelleted at 12,000 × g for 10 min. Viruses were pelleted (78,000 × g, 2 hr for polio and Sindbis; 30,000 × g, 1.5 hr for Sendai) from clarified (10,000 × g, 10 min) culture supernatant fluids. Dextran sulfate (2 μg/ml) was added as a ribonuclease inhibitor prior to pelleting cells or viruses. RNA was prepared from Sindbis virus by suspending the pellets in 1% sodium dodecyl sulfate (SDS) and 2 μg of dextran sulfate per ml in diluted electrophoresis buffer (0.006 M tris(hydroxymethyl)-aminomethane-acetate, 0.001 M ethylenediaminetetraacetate, pH 7.2) for a few minutes at room temperature. All other RNA species, except as noted, were prepared from cell or viral pellets by the phenol-SDS procedure (22, 23) at 60 C. The cell RNA species had typical sedimentation patterns (22, 23) upon centrifugation in sucrose density gradients, with ribosomal RNA peaks at approximately 18 and 28S (16 and 23S for E. coli). The abbreviated viral purification-concentration procedure was adequate for isolation of labeled RNA species from well characterized viruses employed here, even though they were propagated in the absence of actinomycin D. RNA labeled with ^{3}H was stable for several weeks as a precipitate in alcohol at −20 C or for several months in buffer at −70 C. RNA labeled with ^{32}P was sufficiently stable through several radio-half-lives to serve as a marker, but eventually sharpness of peaks was lost, presumably due to radiation damage.

Electrophoresis. The electrophoresis apparatus was similar to that described by Maizel (17) except that continuous buffer circulation was not employed in this study. The gels (2.25% acrylamide, 0.15% ethylene diacrylate) and conditions for electrophoresis at room temperature were essentially those of Duesberg (6, 7; personal communication), a modification of the procedure of Bishop et al. (1). Glass tubes (114 mm long and 6 mm inside diameter) closed at the lower end with a double thickness of dialysis membrane were employed. Gels (2 ml each) were allowed to polymerize at 37 C for 30 to 60 min and were used immediately without preelectrophoresis. An alcohol precipitate or SDS extract of RNA (usually a mixture containing both isotopes) was suspended in diluted electrophoresis buffer containing 25% glycerol. With a Kirk-type micropipette, the RNA sample was carefully layered beneath the upper electrode buffer and on top of the gel. After initial electrophoresis for 10 min at 30 v, the voltage was increased to 60 v (approximately 5 ma per tube). Time of electrophoresis was recorded from the time of increased current and was usually 2.2 to 2.5 hr. A tracking dye, bromphenol blue, was usually run in a separate tube and reached the end of the gel at or just prior to this time.

Slicing of gels. All parts of the slicing apparatus (Fig. 1) are inexpensive, readily obtainable, and easily assembled. It basically consists of a tube which is mounted in an ice-brine bath and which is fitted with a plunger driven by a hand-operated screw. The tube contains the frozen gel to be sliced. Two nuts (not visible in Fig. 1) to accommodate the driving screw were clamped under a block, fastened to the base by two screws. The driving screw with attached crank, shown in the figure and employed in most of the work, was a standard ⅜ by 16 (⅜ inch diameter, 16 threads/inch) size, and 6 inches long. One turn of this screw provides a slice 1.6-mm thick. Screws of other sizes (e.g., ¼ by 20) to provide different slice thicknesses can be readily interchanged by loosening the clamping block. The slicing tube is glass tubing the same diameter as the electrophoresis tubes. It is mounted by
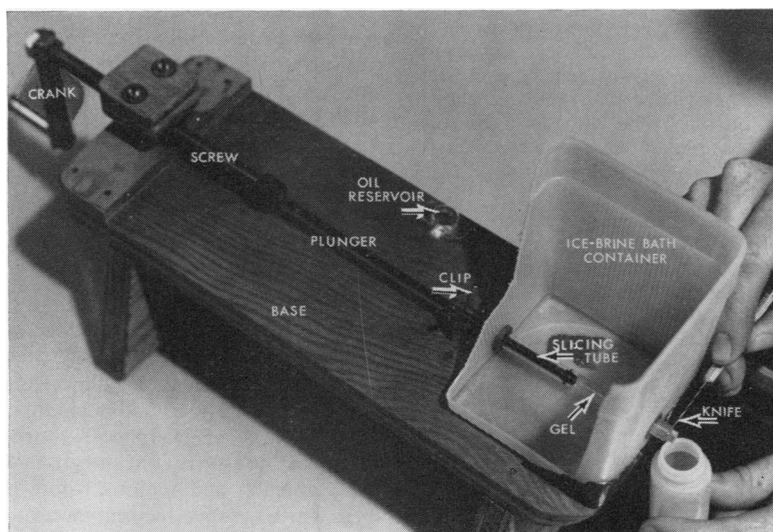

FIG. 1. *Slicing apparatus, showing gel in position for cutting and scintillation vial in position for collection of slice. For photographic illustration, the ice-brine bath was empty and cut away to reveal the slicing tube; an agar gel was substituted for polyacrylamide, and the gel was advanced two turns of the crank rather than the usual one.*

rubber collars in holes bored in opposite sides of a 1.5-pint polyethylene household freezer container which serves to hold a −5 C ice-brine mixture. The front end of the slicing tube protrudes about 7 mm from the container and the protruding rear end is provided with a Tygon tubing collar. The plunger tip (a rubber tip from a 1-ml disposable syringe) closely fits the slicing tube and is mounted on a rod. The other end of the rod fits loosely in a collar on the end of the screw. The unit consisting of bath container, slicing tube, and plunger is held on the base by corner brackets at the front and a clip (two cutoff nails) on the Tygon collar at the rear. It is readily removable from the base. Having several such units available permits sequential loading and cutting of gels. The knife is a Beaver eye knife, handle no. 3K and Lundsgaard Schral blade no. 58, available from surgical supply houses. The oil reservoir is conveniently located for lubricating the knife blade.

At the termination of electrophoresis a "pusher" (short segment of close-fitting glass or Teflon rod) was allowed to settle through the buffer in the upper part of each tube and rest on top of the gel, and the remaining buffer was removed by aspiration. The gel in its electrophoresis tube was rapidly frozen from the bottom end upward in a −70 C alcohol bath, starting with partial immersion followed by deeper immersion with up and down motion. The frozen gel was stored at −20 C until ready for transfer to the slicing tube.

To prepare for receiving the gel, the interior of the slicing tube and the plunger were moistened with alcohol to act as an antifreeze and lubricant. The plunger was positioned flush with the slicing end of the tube, and the unit was chilled in a −20 C freezer. The frozen gel was loosened from the electrophoresis tube by brief immersion in water, and the dialysis membrane was removed. The lower end of the electrophoresis tube was connected to the slicing tube with a short piece of Tygon tubing, butting the two glass tubes together and avoiding entrapped air between the gel and plunger tip. Using a hand-held rod against the "pusher," the frozen gel was gently pushed into the slicing tube far enough so that the entire gel was within the bath container. The unit was returned to the freezer for a few minutes to harden the edges of the gel which may partially thaw during transfer operations.

The slicing unit with its frozen gel was mounted on the base of the apparatus (Fig. 1) and an ice-brine mixture at −5 to −6 C (approximately 1 volume of saturated NaCl solution, 1 volume of water, and 6 volumes of crushed ice) was added to the container. The gel was advanced by means of the screw and plunger to the end of the slicing tube and serial slicing commenced with one turn of the crank per slice. The slices were allowed to drop into scintillation vials when total radioactivity was to be determined or into 2-ml disposable beakers when the RNA was to be eluted. The eye knife blade was lubricated with mineral oil (removing the excess on a cellulose wipe) at the start and once or twice during the slicing operation. The apparatus could be operated efficiently by one person, but for convenience and rapid sectioning (2 to 3 min per gel) it was advantageous for a second person to assist by operating the crank and receiving collection vials. Thus, six to eight gels could easily be frozen, transferred, cut, and collected (45 to 50 slices each) within 1 hr after termination of electrophoresis.

To accommodate large quantities of RNA, gel tubes of 12 mm inside diameter were occasionally employed, increasing the gel volume, sample, eluting buffer, etc. fourfold, and a longer scalpel for slicing was used.

Elution of RNA from gel slices. To each gel slice, 0.5 ml of E buffer (1) was added and allowed to stand overnight at 4 C. The fluid was then sampled for radioassay, and the remaining RNA was recovered from appropriate slices by alcohol precipitation (along with nonradioactive carrier RNA if necessary).

Radioassay. Samples, either gel slices dissolved in piperidine (0.15 ml of 1 M each), or portions of eluted RNA, were assayed in a BBOT-methoxyethanol scintillation fluid (4 g of BBOT, Packard Instrument Co., and 80 g of naphthalene in 600 ml of toluene, plus 400 ml of 2-methoxyethanol) in a Packard Tri-Carb model 4322 liquid scintillation spectrometer. An appropriate correction was applied for ^{32}P spillover into the ^3H channel. In some instances when only ^{32}P was assayed, Cerenkov radiation in aqueous solution was utilized (4).

DMSO treatment of RNA. Treatment of RNA in TE buffer with dimethyl sulfoxide (DMSO) was by the method of Iglewski and Franklin (13).

RESULTS

Evaluation of technique. The design of the gel slicer evolved during attempts to separate cellular and viral RNA species by electrophoresis reproducibly. Frozen gels, under appropriate conditions, were handled with ease. Freezing gels without changing the relative positions of the RNA components was satisfactorily accomplished by carefully freezing from the bottom upward. This caused linear expansion toward the top and eliminated such pitfalls as gel distortion, bursting of the dialysis membrane, and breakage of the gel or tube during freezing. Gels sliced for radioassay in these studies were usually used within 2 days storage, but we believe extended periods of storage at −20 C would be feasible.

Reproducibility of thickness of polyacrylamide gel slices obtained with the apparatus in Fig. 1 was estimated in two ways, by weighing serial slices and by radioassay. In the latter case, ^{32}P in the form of inorganic orthophosphate was added to the upper buffer chamber and allowed to migrate by electrophoresis through the gel to achieve a uniform concentration throughout the gel. Ignoring end regions where the slice thickness varied, reproducibility was quite adequate; in one gel in which both weight and radioactivity were determined, the means and standard deviations for 35 consecutive slices were 42.8 ± 1.5
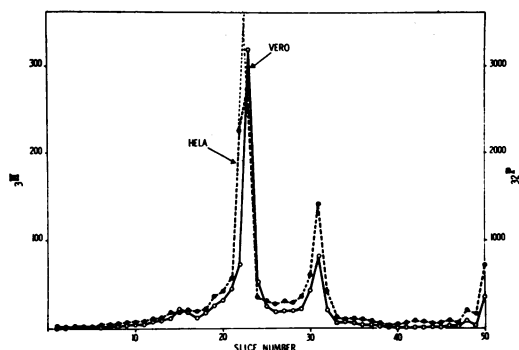
FIG. 2. *Coelectrophoresis of* ^3H-*HeLa RNA and* ^{32}P-*Vero RNA showing partial separation of 28S peaks and coincidence of 18S peaks. Electrophoresis, 138 min; slices, 1.6 mm each. Radioactivity is expressed as counts/minute,* ^3H, ●; ^{32}P, ○. *Position of HeLa 28S RNA peak was estimated by extrapolation. Lower (anode) end of gel is to the right.*

mg and 2,260 ± 147 counts/min, respectively. Exact positions of the gel ends and variations in thickness near the ends were of no importance since relative mobilities were always estimated with reference to two (or more) markers not in the end regions.

An electrophoretic pattern typical of RNA obtained with the procedure described above is shown in Fig. 2. The major peak, representing 28S ribosomal RNA, was well separated from the 18S peak. Radioactivity in the last fraction was due to 4S transfer RNA which had reached the end of the gel prior to termination of electrophoresis and was trapped by the dialysis membrane. The slight asymmetry of the peaks with skewing to the left was due to a factor (probably electrophoretic tailing) independent of the transfer and slicing techniques; this was affirmed by achieving identical patterns with duplicate gels handled in opposite directions.

Molecular weights of RNA. Various combinations of labeled ribosomal and well characterized viral RNA species were employed. These are summarized in Table 1, with the values of Loening (15) for molecular weights of HeLa ribosomal RNA species as standards. Apparent molecular weights were estimated from a straight line drawn through two or more points plotted as log molecular weight versus peak position (slice number) for the appropriate marker RNA species. (When peak activity was obviously distributed in two slices, the peak position was estimated as the intercept of extrapolated slopes of both sides of the peak, as in Fig. 2 and 3.) Vero cell 28S RNA consistently migrated slightly faster than 28S HeLa RNA (Fig. 2), indicating a

lower apparent molecular weight. MDBK cell RNA was indistinguishable from Vero cell RNA, but both 28 and 18S RNA species from chick cells had slightly higher mobilities, and thus lower apparent molecular weights, than the corresponding mammalian RNA species.

The relationship between log molecular weight and relative mobility (slice number) was affirmed as shown in Fig. 3A with Vero and *E. coli* ribosomal RNA species and in Fig. 3B with Vero ribosomal RNA and Sindbis virus RNA. The molecular weight values for Vero cell RNA were taken from Table 1; *E. coli* 23 and 16S RNA species were assumed to be 1.07 and 0.56 million daltons, respectively (15), and Sindbis virus RNA 4.0 million daltons (5, 14). A linear fit was obtained with the bacterial and mammalian ribosomal RNA species, whereas the relationship between Vero cell and Sindbis virus departed slightly from linearity. The latter was typical of viral RNA species. In Table 1 the estimates of apparent molecular weights of viral RNA were obtained by extrapolation from ribosomal RNA peaks. Employing short extrapolations, the fit with TMV RNA, assuming a molecular weight of 2.1 × 10⁶ daltons (3), was somewhat discrepant. On the other hand, the fit with poliovirus RNA, assuming a molecular weight of 2.4 to 2.5 million daltons (9), was quite precise. As might be expected, the long extrapolation to Sendai virus RNA showed poor reproducibility and a poor fit. The value obtained by extrapolation, 5.5 million daltons, was lower than estimates of 6.3 to 7.5 million daltons, assuming comparable molecular weights for Sendai and Newcastle disease virus RNA species (7, 20).

We attempted to detect a molecular weight difference of approximately 40,000 daltons between 28S RNA species prepared by phenol extraction at 60 and 0 C. It has been shown that a small fragment is separated from 28S RNA by hot phenol treatment (2, 19). HeLa and chick RNA species were each prepared at the two temperatures and subjected to electrophoresis in appropriate combinations. No significant difference was detected between hot and cold phenol preparations either in the position of the 28S peak or in isotope ratios of the leading and trailing sides of the peak. Similarly, chick RNA prepared (without heating) by SDS treatment (19) of a cytoplasmic extract did not differ from a hot phenol-SDS preparation.

Recovery of RNA from gel slices and effect of DMSO on ribosomal RNA. Elution of RNA from gel slices by overnight soaking in buffer was virtually complete, based on three criteria: (i) identical patterns from duplicate gels, one of

TABLE 1. *Summary of molecular weight estimations of RNA by gel electrophoresis*

RNA	Apparent molecular weight (million daltons)		Marker	No. of determinations
	Average	Range		
28S ribosomal				
HeLa	1.75		Standard	
Vero	1.67	(1.66–1.68)	HeLa	4
MDBK	1.67[a]		Vero	1
Chick	1.56	(1.52–1.60)	HeLa, Vero, MDBK	8
18S ribosomal				
HeLa	0.70		Standard	
Vero	0.70[a]		HeLa	4
MDBK	0.70[a]		Vero	1
Chick	0.65	(0.63–0.67)	HeLa, Vero, MDBK	8
Viral				
Sindbis	3.8	(3.5–4.1)	Vero, chick	18[b]
Tobacco mosaic virus	2.4	(2.2–2.5)	Vero	3
Polio	2.5	(2.4–2.7)	HeLa, Vero	3
Sendai	5.5	(5.1–5.9)	Vero	2

[a] Peaks were coincident with marker.

[b] Included are determinations in which Sindbis RNA served as a marker in studies with other viral RNA species (*manuscript in preparation*).

which was assayed after solution of slices in piperidine and the other after elution into buffer (correcting for volume), (ii) recovery by elution from half slices (each slice cut longitudinally), compared with the other corresponding half slices dissolved in piperidine, and (iii) radioactivity of gel residue (separated from fluid eluting buffer by centrifugation) no greater than expected from its packed volume.

The preparative technique was adapted to handling and recovering larger quantities of RNA, by employing gels of twice the usual diameter. Unlabeled chick cell RNA was mixed with radioactive Vero cell RNA, and the resulting electrophoretic peaks were determined by absorbance at 260 nm and by radioassay, respectively. The peak positions showed separations identical to those for the chick-Vero combination employing two radioisotopes (Table 1).

As a test for stability of RNA through the recovery operations, Vero RNA labeled with ³H-uridine was subjected to gel electrophoresis, and slices were eluted with E buffer. After counting small portions, the 28 and 18S peak regions were pooled separately and precipitated along with ³²P-labeled Vero RNA marker. A portion of each precipitate dissolved in TE buffer was held in an ice bath, and an equal portion was treated with DMSO. Each sample was precipitated with alcohol again and subjected to electrophoresis. The results obtained with 28S RNA are shown in Fig. 4; the results with 18S RNA

were similar. Figure 4A clearly demonstrates that the recovered RNA was not altered with respect to electrophoretic pattern. Although DMSO treatment (Fig. 4B) altered patterns both of the ³H-labeled 28S fraction and of the ³²P-labeled marker, positions of the major peaks were not changed. In addition to the more heterogeneous distribution of radioactivity after DMSO, the total recovered radioactivity was somewhat less than that of the untreated portion. It is not known whether the lower recovery was due to degradation to low-molecular-weight material or due to failure to recover all of the RNA upon alcohol precipitation. Recovery of undegraded RNA from gel slices and apparent partial degradation of RNA upon treatment with DMSO have also been observed in other experiments with viral RNA as well as with ribosomal RNA.

To substantiate further the capability of electrophoretic fractionation and the slicing technique, separation and recovery of a mixture of chick and Vero ribosomal RNA species bearing the same radioactive label (³H) were successfully achieved by re-electrophoresis. No evidence for double peaks was obtained upon the first cycle of electrophoresis. Two fractions were taken from the 18S peak which had a truncated appearance. Upon re-electrophoresis the slower migrating fraction was coincident with a ³²P Vero RNA marker, whereas the faster migrating component gave a typical chick 18S peak (6.6 × 10⁵ daltons). Three fractions were taken from the

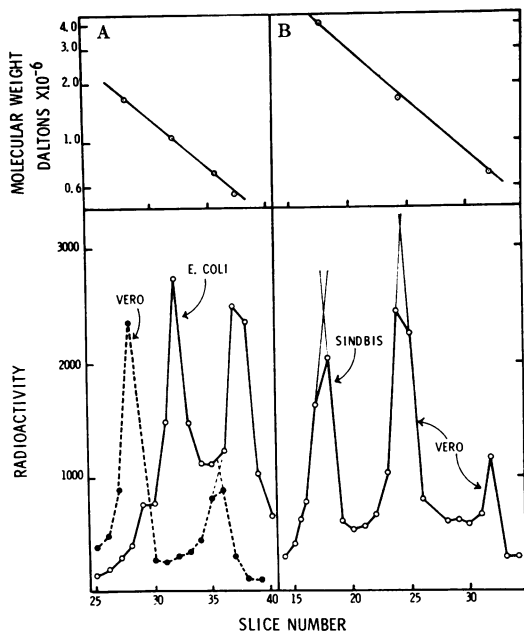FIG. 3. Coelectrophoresis of (A) ³H-Vero RNA and ³²P-E. coli RNA, and (B) ³²P-Sindbis virus RNA and ³²P-Vero RNA. Lower: distribution of radioactivity in gels. Upper: Semilogarithmic plot of molecular weights versus peak positions. Only center portion of each gel is shown; slices, 1.6 mm each. Electrophoresis: (A) 145 min, (B) 150 min (in separate runs). Radioactivity is expressed as counts/minute; ³H, ●; ³²P, O. Positions of peaks were estimated by extrapolation as indicated. Lower (anode) end of each gel is to the right.

FIG. 4. Coelectrophoresis of ³²P-Vero RNA and electrophoretically isolated ³H-28S Vero RNA (A) and effect of dimethyl sulfoxide treatment of the same mixture (B). Only ribosomal RNA regions are shown. Electrophoresis, 136 min; slices, 1.6 mm each. Radioactivity is expressed as counts/minute; ³H, ●; ³²P, O. Lower (anode) end of each gel is to the right.

original 28S peak for re-electrophoresis. The slower shoulder was coincident with the Vero marker, and the faster shoulder was characteristic of chick 28S RNA (1.54 × 10⁶ daltons). The peak fraction, however, recycled as a single peak, with an intermediate apparent molecular weight of 1.62 × 10⁶ daltons.

Similar experiments were employed in an attempt to resolve doublets of 18S RNA reported by Peacock and Dingman (18). Upon recycling, Vero 18S RNA showed only one major component. Minor components were detected from both the leading and trailing shoulders. The identities of these minor components, whether from ribosomes or other cellular RNA species, were not ascertained.

DISCUSSION

The simple, inexpensive device described here is shown to be very practical for its intended application, the electrophoretic analysis of high-mo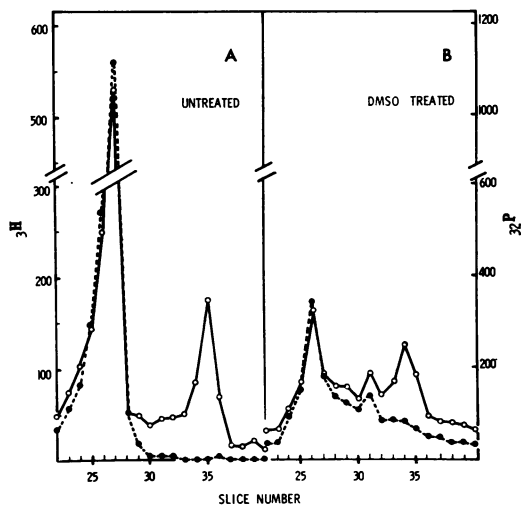lecular-weight RNA in dilute polyacrylamide gels. Mechanically, it is much simpler than devices such as a "macrotome" (10), and a separate cold chamber for proper temperature maintenance is obviated by incorporation of a simple ice-brine bath. The difficulty in removing nearly fluid gels from electrophoresis tubes is circumvented by freezing in situ, an approach also employed by Girard and Marty (8). Gels of 2.2 and 2.7% concentration (acrylamide plus ethylene diacrylate) have been successfully transferred and sliced, as well as the 2.4% gel arbitrarily chosen for this study. Gels of higher concentration, as commonly used for electrophoresis of proteins and of low-molecular-weight RNA, are readily transferred and fractionated in the unfrozen state.

Reproducible results were obtained with replicate RNA samples subjected to electrophoresis at the same time. Identical samples in different runs were somewhat less reproducible with respect to exact positions and shapes of peaks, presumably due to variation in conditions of gel formation and electrophoresis. Nevertheless, the relative positions of peaks in separate runs were highly reproducible, and with the use of known RNA species as markers the relative mobility of any RNA could be reliably estimated. Overloading gels with RNA resulted in trapping at the gel tops and tailing of peaks. Thus, whenever possible, the quantity of RNA applied to 6-mm-

diameter gels was held to less than 100 μg, but as much as 600 μg has been successfully run in 12-mm gels. An excessive concentration of SDS in the layered RNA sample (e.g., undiluted Sindbis RNA or other species prepared by the 1% SDS technique) also caused trapping at the origin and poor resolution of peaks.

The procedure was applicable as a micropreparative technique (employing both 6- and 12-mm-diameter gels) wherein RNA recovered from gels was subjected to further treatment and re-electrophoresis. The same approach served to demonstrate a problem that may arise in the use of DMSO, a reagent valuable for splitting double-stranded RNA (13) and disaggregating high-molecular-weight RNA from tumor viruses (7). The distribution of RNA after treatment was quite heterogeneous when compared with the untreated preparation, but relative mobilities of the major single-stranded components were not altered. Although the treatment itself may have caused some breaks in the strands, it has been suggested that DMSO (or heat) reveals preexisting breaks that were not apparent originally because of tertiary structural integrity of the molecule (7).

Loening (15) estimated the limit of resolution of his method, which employed optical scanning, to be approximately 4% of the molecular weight. The procedure employing two radioisotopes potentially has a higher resolving power. Isolation of peak or shoulder fractions followed by re-electrophoresis with appropriate markers magnifies this resolving power. The approach even enables one to detect heterogeneity within a seemingly homogeneous peak region. Peacock and Dingman (18) reported 18S RNA to be a doublet. Our failure to detect two components may have been due to different sources of RNA or different gel compositions. It is to be noted, however, that most investigators show 18S RNA as a single peak. The failure to detect a small difference in molecular weight between 28S RNA species prepared by hot and cold phenol (or SDS) techniques may be due to a compensatory structural change, as suggested by Loening (15, 16).

Loening (15, 16) electrophoretically analyzed ribosomal RNA samples of numerous species and discussed the relation between molecular weights and evolution. He pointed out that, although absolute values for molecular weights are not obtained by gel electrophoresis, apparent molecular weights can be estimated by comparison with certain RNA species selected as standards. (Molecular weight estimates for the standards were determined by other means.) He further pointed out that at least two markers are required. Loening also discussed possible minor effects of configuration of RNA on relative mobilities. Within this framework, and using the same standards as Loening, we applied our technique to the determination of apparent molecular weights of ribosomal RNA components of cultured mammalian and avian cells. We found a difference in the 18S components of HeLa and chick cells, whereas Loening (15) reported no difference. Since he used chick liver, the possibility of tissue differences, although unlikely, cannot be ruled out. Our value for chick 28S RNA, 1.56×10^6 daltons, is in agreement with Loening's value of 1.58×10^6 (15). Loening observed values of 1.71×10^6 and 1.72×10^6 for mouse and rabbit 28S RNA species, as estimated from an *E. coli* standard, but did not consider them significantly different from HeLa cell 28S RNA. On the other hand, we found 28S RNA species of two nonhuman mammalian cells, monkey and bovine, indistinguishable from each other, with an apparent molecular weight value of 1.67×10^6 daltons when compared with human (HeLa) cells at 1.75×10^6. Additional comparisons, made with best resolution attainable, will be needed to enable one to categorize the various mammalian species and to decide whether human cells in general, or HeLa cells specifically, are unique in electrophoretic behavior of their 28S RNA.

The relation between log molecular weight and relative mobility (1, 15–18) was valid in the 0.5 to 4 million dalton range (Fig. 3 and Table 1). A peak of 4S transfer RNA observed in some runs of short duration (ca. 2 hr) did not fit the plot. This may have been due to deformation of the lower end of the gel, but it is likely that the gel concentration, 2.4%, was too low for resolution in the low-molecular-weight range. Slight discrepancies in the relation between ribosomal RNA and various viral RNA species were observed by Bishop and co-workers (1). Our results, including some different viruses and extension to higher molecular weights, also showed slight discrepancies, that of TMV RNA being the most pronounced. We obtained a much better fit with poliovirus RNA than did Girard and Marty (8), who used a somewhat different gel system. Our results with Sindbis RNA were quite comparable to findings in acrylamide-agarose gels (5, 14). The results with Sendai RNA and Duesberg's (6, 7) with Newcastle disease virus RNA suggest that the relation, though perhaps not linear, may be extended to the 6- to 7-million dalton region and even beyond. Such an extention must await more precise determinations of molecular weights of RNA species to serve as standards in these

regions. To be of greatest value in estimating molecular weights of RNA species of other viruses, coelectrophoresis with a well characterized marker of similar molecular weight should be employed and long extrapolations avoided.

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